NUTRITION REVIEWS

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THE FOOD PROBLEMS OF FREE CHINA

The food problems of Free China are those of prewar China greatly accentuated and augmented by the six years of war. Nutritional surveys and clinical studies made in various parts of China during the years immediately preceding the Japanese invasion in 1937 disclosed grave dietary and nutritional inadequacies as well as malnutrition among many groups of people. These included xerophthalmia, night blindness, urinary calculus, hyperkeratosis, osteomalacia, rickets, tetany, avitaminosis C, ariboflavinosis, beriberi, pellagra, as well as nutritional edemas and anemias. In many instances the deficiencies were multiple. Further clinical studies made in East China during the four years from 1937 to Pearl Harbor revealed startling increases in the incidence as well as in the severity of these manifestations of malnutrition especially among the refugees.

In Free China many of the newer problems are directly connected with the vast migration of tens of millions of people from East China into a territory often already densely populated. These refugees must be fed until fully restored to health, rehabilitated and reestablished on the land as food producers. Furthermore extra burdens are placed on the farmers to provide for the fourteen million or more men in the various branches of China's armed forces, a large proportion of whom have been taken from the farms. The scorched earth policy has further reduced the food available in areas near the battle line such as the rich rice growing districts around Changsha. Inflation has raised the cost of living index to an unprecedentedly high level of 6000 per cent or sixty times the 1937 prewar level. The resulting impossibly high cost of foods has so impoverished the professional and salaried groups that they are now suffering from serious widespread malnutrition and a generally lowered resistance. The increase in general morbidity and especially in tuberculosis is tragic.

Those who would assist in solving Free China's food problems must bear in mind (1) the distressing spread of poverty among the people which now reaches into the middle classes as never before; (2) the inadequacy of the quality as well as the quantity of the food in many sections; (3) the almost complete absence of milk and of dairy products in the diets; (4) the meager and undeveloped methods of transportation in many parts of the country; and (5) the total absence of refrigeration for perishable articles in transit as well as for the storage of food in the average community. Furthermore, inflation with its rocketing prices for rice and other grains has resulted in farmers selling for cash grain that should be used for feeding farm animals. This has brought about a decrease in the actual production of meats, poultry, and eggs. These articles are more scarce today than they were before the war. The universal necessity for cooking almost all fresh vegetables before consumption leads to serious vitamin losses and increases the problem of maintaining the heat labile vitamins at an adequate level.

China's food problems cannot be adequately met if we assume that they are the same in all parts of the country. The basic food crops grown and the diets eaten differ fundamentally in the several sections of the country according to the topography of the land, climatic conditions, soil formations, cultural traditions, food habits, and the total effects of the human impacts on the face of this ancient land where huge populations have succeeded in maintaining a

permanent agriculture for more than forty centuries. Of course there are common factors in the different food producing areas but any long range plan for solving China's nutritional problems must give detailed consideration to the particular factors in each major food producing area which have given rise to the special problems of that specific area.

More than 85 per cent of the people live in rural regions or in cities which must depend primarily on the foods grown in surrounding districts, so we may well use China's nine agricultural areas as the basic areas for studying her food problems. Three of these areas are now temporarily under the control of the Japanese invaders so any current consideration must of necessity be confined to the six areas now in Free China.

A comprehensive picture of the diets of each of these areas may be obtained from the above table compiled from the dietary surveys made by J. Lossing Buck and his colleagues of the University of Nanking during the years 1929 to 1933.

It is obvious from the above table that these diets are bulky, with cereals and legumes supplying from 81 to 95 per cent of the total calories. In comparison with typical American diets we note the almost complete absence of milk and dairy products; the low intake of animal products; the surprisingly small role which soybeans play in the diets; the rather low intake of vegetables; the still lower percentage of fruits and the very minor share which sugar contributes.

Additional data from the above and other surveys in Free China indicate (1) a low protein intake of inferior quality; (2) a low calcium intake varying from 329 mg. to 459 mg. per adult male unit per day in each of the above areas; (3) an unsatisfactory calcium-phosphorus ratio ranging from 1:4 to 1:6 in the above areas (in one locality it

The major food producing areas of Free China and the percentage distribution of the calories supplied by different classes of foods in the farm family diets of each area

Major food producing areas	Chief food crops grown	Seeds				Vegetables	Fruits	Vegetable oils	Animal products		Sugar
		Cereal grains	Legumes		Total seeds				Meat, fish, poultry, eggs	Dairy products	
			Soy beans	Other legumes							
Northwestern area	Millet, spring-wheat, oats, Irish potatoes, kaoliang	82.2	1.1	5.4	88.7	9.1	0.1	1.0	1.1	*	*
North Central area	Winter-wheat, millet, corn, kaoliang, legumes	86.7	3.1	5.3	95.1	2.4	0.4	1.4	0.6	*	0.1
West Central or Szechwan area	Rice, corn, legumes, wheat	79.6	1.9	4.9	86.4	5.9	*	1.3	5.6	*	0.8
Central area	Rice, wheat, barley	85.4	0.9	2.1	88.4	4.8	*	3.3	3.0	*	0.5
Southwestern area	Rice, corn, legumes	84.5	1.4	3.2	89.1	3.2	0.3	0.6	5.8	*	1.0
Southeastern area	Rice, sweet potatoes	79.3	0.6	1.7	81.6	9.8	0.1	4.4	3.1	*	1.0

* The amount is under 0.05%.

was 1:9); (4) a serious iodine deficiency in some districts of Yunnan where 25 per cent or more of the people have simple goiter; and (5) substandard iron intakes in several localities.

Medical observations in Free China during these war years add further evidence of widespread manifestations of malnutrition arising not only from inadequate intakes but also resulting from secondary or conditioning factors such as unusual physical exertion, pregnancy, lactation, or certain types of illnesses which increase the requirement, impair the absorption, or hasten the excretion of the essential nutrients. In all these areas the individuals who suffer most from malnutrition are pregnant and lactating women, infants, growing children, and convalescents, especially wounded or disabled soldiers.

It is clear that China has tremendous food problems. These will undoubtedly be increased when her armies push back the invaders who will probably destroy all standing food crops and stored grains as they retreat. These problems are a challenge to the Chinese government and we can assume that under the National Health Administration and other government agencies China's highly trained scientists are already at work on a long term program to remedy the situation. The projects will undoubtedly include (1) a greatly increased production of the leafy vegetables and fruits most suited to each area; (2) an augmented production of soybeans, soybean milk, curd, and sprouts; (3) improvement in animal husbandry leading to a much greater production of eggs and to the development of a dairy industry using the milk of cows, goats, and water buffaloes; (4) a study of the advisability of adding a calcium salt such as calcium carbonate to ordinary table salt; (5) stocking of rural China's thousands of ponds with such types of fresh water fish as will contribute most to the supply of vitamins A and D in the diets; (6) commercial production of yeast as a food; (7) addition of potassium iodide to the table salt consumed in all the goitrous districts; (8) adequate protection of all grains in storage and regulations restricting the sale of highly polished rice; (9) establishment of food and nutritional research programs in each major area, adequately staffed with trained medical personnel as well as nutritionists so that special attention can be given to the clinical observations on mothers and children of all ages; and (10) an educational program which will be effective in changing the food habits of the people so that they will profit by using the better foods.

It is to be hoped that America can make her contribution to the immediate needs of our allies by increasing the air shipments to Free China of fish liver oil concentrates, vitamin B-complex, and ascorbic acid for use in her hospitals and other institutions under medical supervision. The problem of caring for infants, mothers, and young children will be greatly aided by large shipments of powdered whole milk the instant truck transportation is available over the Burma Road. Some of the protein deficiencies may also be alleviated by similar shipments of dehydrated pork and other meats.

The endurance of the Chinese people through six years of war has called forth the admiration of the entire world. By contributing to the solution of their urgent food problems now we can translate our admiration into action which will mean health and life for millions.

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HUMAN AMINO ACID REQUIREMENTS

Our knowledge of the amino acid requirements of man is fragmentary compared to that of the rat which has been so carefully developed by the work of Rose and associates at the University of Illinois. This important subject is now under investigation both by Rose and by Holt and coworkers at Johns Hopkins University. Holt *et al.* (*Proc. Soc. Exp. Biol. Med.* **48,** *726, 728 (1941)*) have already indicated that tryptophane and lysine are required in the diet of man, by demonstrating that nitrogen balance cannot be maintained when these amino acids are lacking in the food. They have also shown that arginine is essential for normal spermatogenesis, (*Fed. Proc.* **1,** *116 (1942)*).

More recently the observations on lysine deficiency have been significantly augmented by the demonstration that certain symptoms and a biochemical defect occur during the deficiency (Albanese *et al.*, *Proc. Soc. Exp. Biol. Med.* **52,** *209 (1943)*). Three subjects fed a lysine deficient diet all complained of nausea, dizziness, and hypersensitivity to metallic sounds on the fifth day of the regimen. No objective clinical signs accompanied the development of these symptoms, but a sharp rise occurred in the urinary excretion of non-ketone organic acids. The nitrogen balance became negative. A lysine containing diet was substituted and within four days all symptoms disappeared, the organic acid excretion fell to normal, and nitrogen equilibrium was restored. In subsequent experiments it was noted that the rise in urinary output of non-ketone organic acids was sometimes delayed and the symptoms mentioned did not appear regularly. The abnormality of organic acid excretion appears to be a biochemical lesion characteristic of lysine deficiency. It is not related *per se* to a negative nitrogen balance and it was not encountered in single amino acid deficiencies of tryptophane, methionine, and cystine. The authors point out that since increases of non-ketone organic acids in the urine have been found in pneumonia, nephrosis, malnutrition, postoperative acidosis, premature infants, and in rickets, it is at least possible that an abnormality of lysine catabolism may be present in these states.

The most recent report from the Johns Hopkins group (*Proc. Soc. Exp. Biol. Med.* **52,** *18 (1943)*) demonstrates that methionine and possibly cystine are also essential to normal human nutrition. Nitrogen balance studies were conducted on 2 male and 1 female subject whose diets consisted of fats, sugar, and certain fruits and vegetables selected for their low nitrogen content. It was estimated that approximately 10 per cent of the nitrogen requirement was thus supplied, the remainder being provided by an amino acid mixture. This mixture was prepared from acid hydrolysates of casein and treated so as to be deficient either in methionine or cystine. The methionine deficient preparation was fortified with cystine, and tryptophane was added to both diets to replace that destroyed by autolysis. The subjects were given enough of this mixture to maintain their nitrogen intake at 0.1 g. per kilo per day. The remaining foods were not adjusted to the individual's weight, hence the lighter subjects received more calories per kilogram than the heavier ones. A control period of four days preceded and another of eight days followed the deficient period, during which time the ration was supplemented with the amino acid under test.

During the methionine deficiency period all 3 subjects developed a negative nitrogen balance and lost weight, an effect which was reversed upon addition of methionine to the diet. The female subject developed a relatively small negative balance during the deficiency period, but it was pointed out that she weighed less than the male subjects and that the relatively larger amounts of carbohydrate and fat given her may have

had some protein sparing effect. These experiments clearly indicate that methionine may be regarded as a dietary essential for man.

The experiments with the cystine-poor diets were inconclusive. The female subject maintained her weight and remained in nitrogen balance throughout the period. Although both men lost weight, only one clearly showed a negative nitrogen balance. Both subjects showed a slight increase in nitrogen retention when cystine was added during the second control period, but the changes were small and indefinite. These observations, therefore, failed to demonstrate whether or not cystine is an essential amino acid for man.

A preliminary report by Rose, Haines, and Johnson (*J. Biol. Chem.* **146,** *683 (1942)*) provides proof that the amino acids valine and methionine are indispensable dietary components for man. These investigators used 2 healthy young men as subjects. Their diets were not reported in detail, but from the standpoint of nitrogen content appear to consist almost entirely of a mixture of pure amino acids. Caloric intakes were adjusted by carbohydrate content to be in exact proportion to the subjects' weights. Known vitamins were supplied daily in pure form and "unidentified" factors as a concentrate of liver equivalent to 5 g. of Wilson's "liver powder 1:20." Preliminary experiments showed that 5.66 g. of nitrogen daily was not sufficient to maintain nitrogen balance, but 7.02 g. daily readily established equilibrium. The fact that nitrogen equilibrium was so readily established on this low level of nitrogen supplied as the ten amino acids previously shown to be indispensable to rats, led the authors to the conclusion that the other twelve known amino acids are dispensable to man.

After an initial maintenance period of eight days, valine was removed from the ration and the other amino acids increased to provide a constant nitrogen intake. The subjects immediately developed a marked negative nitrogen balance which could be reversed by replacement of valine in the diet. A similar though less pronounced effect was found in a comparable experiment with methionine.

The latest report from Rose's laboratory (*J. Biol. Chem.* **148,** *457 (1943)*) shows in the same manner that threonine, leucine, isoleucine, and phenylalanine are necessary constituents of the diet. Removal of histidine from the diet, however, did not cause a negative nitrogen balance. This was an unexpected finding since this amino acid had been early established as essential to the rat. The other amino acids were tested for possible contamination with histidine but with negative results. The negative balance produced by isoleucine or phenylalanine was promptly changed by the addition of these amino acids to the diet, even when histidine was absent. It is therefore concluded that histidine is not required in the diet of man for nitrogen equilibrium.

The investigations of Rose leave only the testing of tryptophane, lysine, and arginine. Of the ten amino acids previously shown to be essential in the diet of the rat, six—valine, methionine, threonine, leucine, isoleuine, and phenylalanine—are also "indispensable" to man. One—histidine—appears not to be required by man. The remaining three, lysine, tryptophane, and arginine, have been found by Holt and associates to be essential, arginine at least for normal spermatogenesis.

This most important phase of human nutrition has developed readily, and it is significant that such rapid progress is made possible only by years of careful animal experimentation.

A NEW METHOD FOR VITAMIN C DETERMINATION

Most chemical methods for the determination of ascorbic acid have depended on its reaction with 2,6 dichloro-benzenone-indophenol, in which only the reduced form of the vitamin reacts with the dye. It is possible but not a satisfactory procedure to reduce the dehydroascorbic acid with hydrogen sulfide and to titrate the ascorbic acid formed.

A new analytical method has been described by Roe and Kuether (*J. Biol. Chem.* **147,** *399 (1943)*) in which reduced ascorbic acid is oxidized and the dehydroascorbic acid measured. It has been described thus far only for blood and urine and cannot yet be assumed to be applicable to food products. The method should be of particular interest to those engaged in studying the vitamin C status of individuals by means of blood studies. Vitamin C determinations have in the past been done mostly on plasma since the liberation of oxyhemoglobin from red blood cells during deproteinization of whole blood oxidizes the reduced ascorbic acid. In some methods this oxidation has been prevented by "stabilizing" the hemoglobin by saturation with carbon monoxide, but such measures are not practical in clinical procedures. It is known that ascorbic acid disappears more rapidly from plasma than from red blood cells, white blood cells, or platelets. Determination of ascorbic acid in whole blood would therefore be of greater significance than plasma measurements in the evaluation of more severe degrees of deficiency.

In the new method, whole blood is deproteinized with trichloracetic acid. The filtrate is shaken with norit charcoal which under the conditions insures complete oxidation of the ascorbic acid to dehydroascorbic acid. The color reaction carried out on the norit filtrate depends on the formation of a colored 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid in the presence of sulfuric acid. Thiourea is added to counteract the effect of oxidants which had been found to cause slight coloration of the 2,4-dinitrophenylhydrazine.

The method is claimed to be sensitive to 0.2 micrograms of ascorbic acid in the 4 ml. of filtrate used for analysis. Statistical evidence is presented in support of the authors' contention that the method is highly accurate and specific. Keto compounds such as pyruvic acid and acetoacetic acid do not interfere. Pentoses, glucose, fructose, or glucuronic acid interfere slightly but not at the concentrations which normally occur.

The color is stable for forty minutes and after standing for eighteen hours the maximum fading amounts to only 2 points on the galvanometer. Large numbers of determinations may therefore be run simultaneously. The authors include a calculation chart in which galvanometer readings of an Evelyn photoelectric colorimeter are recorded against milligrams of ascorbic acid. The filter recommended is one which transmits maximally at 540 millimicrons. Additional instructions are included in case a null point type instrument is used. Detailed instructions are given relative to necessary precautions and for the dilutions at which most satisfactory results can be obtained. It is necessary to use a photoelectric colorimeter in order to compensate for the yellow color in the reagent blank.

Ascorbic acid in whole blood of acutely scorbutic guinea pigs ranged from 0.04 to 0.13 mg. per 100 ml. and urine values from 1.0 to 3.0 mg. per liter.

This method, due to its apparent accuracy, specificity, precision, ease of serial analyses, and its application to whole blood, should find considerable use.

GROWTH FACTORS IN INJURED CELLS

The rapid growth and proliferation of cells at the site of an injury has long attracted attention and several investigations have indicated the release of growth or proliferation stimulating materials from the injured cells. Since the phenomenon of growth has long been utilized as a measure of nutritional efficiency, it would not be surprising if such growth stimulating factors were identical with some of the known nutritive factors such as the vitamins.

Loofbourow (*Biochem. J.* **36,** *737 (1942)*) has published evidence to show that yeast cells, upon damage with ultraviolet radiation, excrete growth promoting substances into the fluid in which they are suspended. His data indicate specifically that slightly damaged cells elaborate certain factors which (1) increase all permeability, (2) cause diffusion of nonprotein substances through the cell membranes, and (3) synthesize within the living injured cells a variety of nonprotein growth stimulating substances. Some of these effects may be correlated with the edema, profuse blood supply, and the swelling that accompany the early phases of healing in animal tissues. The edematous fluid may carry the growth principles which hasten repair.

Experimentally, Loofbourow injured yeast cells by submitting them to sublethal doses of ultraviolet light. Examination of the cell suspensions of normal and damaged yeast cells showed several differences. The fluorescence produced by radiation with light of 3650 Å was blue for the normal cells, but yellow-green for the damaged cells. The fluid in which the normal cells were suspended contained 0.245 mg. of solids per ml., while that from the injured cells contained 3.16 mg. Tests for proliferative activity of these fluids gave values of 3.3 growth units per ml. for the normal and 98 growth units for the fluid from injured cells. (One growth unit represents an increased twenty-four hour yeast crop of approximately 1000 per cent in comparison with the controls.) Only 10 per cent of the activity of the growth stimulating substances was lost during autoclaving. Ultraviolet absorption studies and solvent fractionation indicated the presence of nucleotides, but apparently all the activity was not accounted for by this fraction.

The growth stimulating effects of two groups of known growth factors were studied. The first contained members of the vitamin B-complex, namely thiamine, riboflavin, pyridoxine, calcium pantothenate, biotin, choline, and nicotinamide. The second group of substances contained the amino acids, glutamic acid, asparagine, aspartic acid, leucine, and arginine. The damaged cell products however caused an increased growth of yeast even when the medium was already supplemented with these vitamins and amino acids. The addition of adenosine triphosphate, muscle adenylic acid, and adenosine had a similar effect, but only a combination of the B-vitamins and adenosine triphosphate completely replaced the products developed by the injured cell.

Previous work has indicated that the general phenomenon of the liberation of growth stimulating substances may be independent of the type of injury or tissue. It would therefore be desirable to know, from the practical viewpoint, whether the use of adenosine, B-vitamins, or concentrates of the exudate of damaged tissue might have beneficial effects on wound healing and tissue regeneration.

B-VITAMIN DEFICIENCY AND THERAPEUTICS

The symptoms and signs of early nutritional deficiency in children are notoriously unspecific. The discussion of Holt (*South. Med. Surg.* **103,** *9 (1943)*) makes it clear that these clinical criteria (lack of appetite, failure to gain weight, aversion to play, poor muscle tone, etc.) leave the physician in a therapeutic dilemma. It is true that the administration of vitamin B- complex either therapeutically or as a preventive measure is not harmful except to the patient's pocketbook, although it is pointed out that administration of single members of the vitamin B-complex may precipitate more severe symptoms of another latent deficiency.

The logical solution to the physician's dilemma is to have available more specific symptoms of mild deficiency or adequate laboratory criteria. Holt and his associate Najjar have been engaged in providing simple procedures for detecting deficiencies of thiamine, riboflavin, and nicotinic acid. The body reserves of these three vitamins are said to be reflected in their urinary excretion. Thiamine is detected in the urine by the fluorescence of its derivative thiochrome, and riboflavin by its own fluorescence after extraction. In the case of nicotinic acid, a fluorescent derivative called F2 may be detected. The qualitative finding of any of the three is said to indicate that the patient is not suffering from any of these deficiencies; the failure to detect any one indicates a lowered reserve and the need for therapy.

The urine collected for analysis must, however, represent the reserve or excess of the vitamin and not simply a reflection of a recent meal. Holt and Najjar have shown that these vitamins may appear in the urine even in deficient subjects after a single vitamin-containing meal, but that this effect is over in eight hours. The procedure adopted, therefore, is to discard the urine during a twelve hour (overnight) fast, and collect a "thirteenth" hour urine sample for fluorometric analysis. The determination of sufficiency or deficiency is based on extensive data in the case of thiamine, but on limited data for riboflavin and nicotinic acid. The tests appear to offer no great technical difficulties, though as yet detailed descriptions of them are not given.

Holt has not made extensive surveys with the method, but one of the most impressive findings is that 9 out of 10 cases of diarrhea in children are accompanied by a thiamine deficiency. Other situations which are said to predispose to deficiency are (1) an unbalanced diet which, often composed predominantly of refined carbohydrate, not only increases the B-vitamin requirements but diminishes the supply of these vitamins, (2) various disturbances of digestion which may interfere with the absorption of the B-vitamins, (3) circulatory disturbances which may interfere with distribution of the vitamins, and finally, (4) various conditions such as fever, hyperthyroidism, pregnancy, and lactation which increase the requirement for these vitamins.

Holt and Najjar, using their "fasting one hour excretion test," have come to the conclusion that the National Research Council recommendation of 1.5 mg. of thiamine daily for the adult is considerably too high. They placed 12 male subjects on a diet consisting of casein, lard, and sugar supplemented by a vitamin and mineral mixture. The thiamine intake was progressively reduced until the "one hour excretion test" reached zero, the end point of the decrease being the point at which the excretion of thiamine was zero about half of the time. The thiamine intake at or during this period varied from 0.375 to 0.625 mg. per day. None of the subjects showed any signs of thiamine deficiency; though it is not clear just how long the subjects were kept on the low thiamine diet it appears to have been about five months. The authors report that the thiamine requirement was influenced by

variations in temperature. It is known that the requirement varies with the amount of exercise and the proportion of carbohydrate in the diet. In passing, it is of interest that an increase of thiamine excretion occurred in hot weather, which, according to Holt's interpretation, indicates greater body stores or lessened requirement. This is in contrast to the findings of Mills (*Am. J. Physiol.* **133,** *525 (1941)*) which indicated an increased thiamine requirement for the rat at higher environmental temperatures.

Holt believes that the actual thiamine requirement of sedentary individuals may be only one third of the amount recommended by the National Research Council. However, all studies of vitamin requirements must take into consideration, in addition to factors of exercise, environmental temperature and caloric intake, the criteria which the investigator uses as evidence of sufficiency. The condition of zero fasting excretion has not yet been correlated with tissue vitamin levels, nor these levels with optimum tissue function of the vitamin.

The average physician cannot consider all the details of what constitutes an adequate or inadequate vitamin intake. He might become more conscious of the role of nutrition in his practice, however, if he could obtain a simple "yes or no" answer to the question of the vitamin status of his patients. The simple laboratory approach suggested by Holt for these three B-vitamins may, therefore, become valuable in the practical diagnosis of vitamin deficiency.

THE GOITROGENIC ACTION OF CALCIUM SALTS

Search for the causative factor in the development of goiter has resulted in a number of experimental studies. Calcium has been suggested as a goitrogenic agent by several investigators and the interrelationship between calcium metabolism and the structure of the thyroid gland is a current study. There has been no general agreement on the role which calcium plays. Surveys by Orr and by Stott showed that goiter often occurs in limestone regions. Thompson found that thyroid size varied directly with the calcium concentration of the diet but Levine and associates report no effect with the same basal low iodine diet. While both Hibbard and Mahorner obtained goitrogenic effect from calcium chloride, Hibbard considered that it was the chloride and not the calcium which caused the thyroid enlargement. In contradiction, Remington concluded from his experiments that chloride was not the factor (1937).

The experimental goiter observed in nearly all reports has been of the hyperplastic type. However, Hellwig asserted that while a hyperplastic type goiter developed when calcium was added to a diet low in iodine, a colloidal goiter resulted when the iodine level was increased.

Sharpless, Sabol, Anthony, and Argetsinger (*J. Nutrition* **25,** *119 (1943)*) contend that it should be possible to demonstrate goitrogenic effect if the basal diet used were one known to produce a slight thyroid enlargement. In their first series of experiments the effect of three calcium salts—chloride, phosphate, and lactate—was studied for changes in thyroid weight and iodine content and concentration. The differences which resulted in this series were insignificant by mathematical analysis. High calcium intake did not affect the size of the gland nor its iodine content. Examination of the glands from each group of rats showed no real differences in the microscopic structure. The thyroid weight, per 100 g. of body weight, was 17 mg., which is approximately twice the normal thyroid weight but only one-half the weight obtained when the diet is not supplemented with iodine.

In the second series the additive influence of vitamin D was tried. Sharpless *et al.*

comment on the report of Abelin that calcium decreases the metabolic effect of thyroxine and therefore should increase the requirement. If calcium should act in this manner, then vitamin D, through its action on absorption, should be as important as the calcium intake. Neither large nor small amounts of vitamin D added to the basal diet had any effect, nor did calcium carbonate, either alone or with 2 per cent viosterol. With calcium chloride, however, the average thyroid weights (per 100 g. of body weight) of rats fed the diet with either 15 drops or 2 per cent viosterol were 20 mg. and 22 mg., respectively.

While 15 drops of viosterol per kilogram of diet had no effect on serum calcium, 2 per cent viosterol raised the average level from 12.6 to 13.3 mg. per 100 ml. The collective facts would indicate that the elevation of the serum calcium in itself did not increase thyroid weight but that in the presence of vitamin D, calcium chloride had significant goitrogenic effect. This confirms the work reported in 1933 by Hibbard that calcium chloride, but not other calcium salts, produces goiter, but not the conclusion that it was the chloride that had the action.

When thyroid enlargement is present and an increase in iodine brings about a normal gland, the accepted explanation is that a relative iodine deficiency originally existed. The iodine content of the gland is therefore a reliable indication of iodine balance. In animals fed the basal diet containing a small amount of iodine, the iodine content of the thyroid gland ranged from 1.6 to 2.3 micrograms. The lowest values, 1.6 and 2.0, were found in those animals receiving the calcium chloride supplement, the lower value being in those with the higher viosterol level. Microscopic examination showed that within the range of 1.6 to 2.3 micrograms the gland presented a normal appearance. Chlorides apparently tend to decrease the concentration of iodine in the gland, perhaps by causing an elimination of iodine before it can be stored. The authors state that other data, not presented in this paper, also indicate that chloride can cause a reduction in the thyroid.

Hellwig's hypothesis (*Arch. Path.* **19,** *364 (1935)*) that goiter is caused by a positive factor along with an iodine deficiency is partially supported by these results, namely, that an apparent decrease in available iodine occurred when calcium chloride produced thyroid enlargement. Because calcium chloride was the only salt that was goitrogenic, neither of its components should be classed as having this property. Furthermore, the compound should be considered as only a potential goitrogenic agent.

Further studies of Sharpless and associates (*J. Nutrition* **25,** *239 (1943)*) report the effect of chloride supplements to diets of both high and low iodine content. The same general procedure as in previous experiments was followed except the use of a second basal diet with a high iodine level. While in all studies on experimental goiter one of the prerequisites is to lower the glandular iodine concentration, it was felt that possibly a diet containing a considerable amount of iodine might show more clearly the tendency of the chloride to lower the glandular iodine concentration. Accordingly, one basal diet contained 4 micrograms of potassium iodide per 100 g. and the other 50 micrograms. A diet containing 4 micrograms is assumed to result in an iodine deficiency inasmuch as a higher iodine level reduces the thyroid weight. The low level offers, however, some protective action since when the iodine supplement is omitted, the thyroid weight is markedly increased. The chlorides were added as the sodium and ammonium salts, hydrochloric acid, and as a combination of calcium chloride and hydrochloric acid.

In the series using the high iodine basal diet the iodine concentration in the glands of the rats receiving no chloride supplement was 0.168 per cent of the dry weight or a total of 5.3 micrograms, while the inclusion of 2.1 per cent sodium chloride reduced this to 0.095 per cent or to 3.6 micrograms total.

There was a slight but insignificant increase in thyroid weight from 11 to 13 mg. per 100 g. of body weight.

Supplements of hydrochloric acid alone or in combination with calcium chloride resulted in a considerable decrease in the iodine concentration but had no effect on thyroid weight.

The iodine concentration in the blood, expressed as micrograms per 100 ml. was: basal 3.7; basal plus 50 micrograms of iodine, 10.5; basal plus 50 micrograms of iodine and 2.1 per cent sodium chloride, 4.1.

The results consistently show that the thyroid iodine concentration can be decreased by feeding an excess of chloride. This decrease can be more clearly demonstrated when the iodine content of the diet is high.

It has long been recognized in goiter studies that a relative iodine deficiency is the most important predisposing factor in the development of the goiter. These studies indicate that positive factors may also influence the utilization of iodine or increase the requirement for thyroxine.

RIBOFLAVIN METABOLISM

Interesting facts concerning riboflavin metabolism have been recently reported. Ellis, Zmachinsky, and Sherman (*J. Nutrition* **25,** *153 (1943)*) present data indicating that the level which seems to support adult vitality and length of life in experimental rats is somewhat less than the amount from which the offspring derive optimum benefit. As in their previous experiments, matched lots of rats were placed on experimental diets at the age of 28 days—the only variable being the riboflavin content which was 3, 6.5, and 10 micrograms per gram of food mixture, or 0.9, 2.0, and 3.0 micrograms per kilogram-calorie of food, respectively. Observations were made on the body weight curve, length of life, reproductive cycle, concentration of hemoglobin in the blood, average weight of offspring on the twenty-eighth day, and on the offspring's weight curves. Analysis of the data indicates that on diet I, at a level of 3 micrograms per gram of dry food or 0.9 micrograms per kilogram-calorie of the diet, the optimum effect was reached. Higher levels had no advantage. This is essentially the same level of riboflavin, relative to total calories of food, as the "Recommended Daily Allowances" (0.9 mg. riboflavin per 1000 calories).

In 1935, Ellis and Bessey suggested that riboflavin was one of the factors contributing to the maintenance of a normal hemoglobin level, and in 1938, György, Robscheit-Robbins and Whipple showed that regeneration of hemoglobin was hastened by dietary riboflavin. In the experiment here reviewed, Ellis and co-workers found that the riboflavin level in diet I "supported the full plateau value of hemoglobin concentration in the blood."

While this level proved optimum for the adult rat, the threefold higher intake level of 10 micrograms per gram of dry food resulted in added benefit to the young. This effect was especially evident when the young rats were transferred to a diet deficient in thiamine, but further study is necessary to determine which factors may be operative in causing the difference in need of adult and young animals.

Little information is available regarding the excretion of riboflavin into the intestine or its absorption from different parts of the gastrointestinal tract. This is not surprising since it is difficult to tell what part of the riboflavin present in the alimentary canal has been synthesized by the intestinal flora and what part is contributed by excretion through the intestinal epithelium. An attempt was made by Selye (*J. Nutrition* **25,** *137 (1943)*) to obtain information on these points by determining the presence of

riboflavin crystals in various parts of the gastrointestinal tract following administration of the compound by the intravenous route, or after direct introduction into isolated intestinal loops.

Previous experiments had shown that if a saturated solution of riboflavin in physiologic saline is injected intravenously into the rat the greater part of it reappears in the urine. Therefore, in order to make other ways of excretion more readily detectable, the rats were bilaterally nephrectomized. Adult male rats weighing from 130 to 170 g. were used. In the first series the intestinal lumen of half the animals was occluded by placing a ligature between the jejunum and ileum. Immediately after injection of 7 mg. of riboflavin the skin of all animals exhibited the characteristic yellow color; five hours later, the skin of the animals with intestinal ligature was still yellow; the others had returned to normal. This was interpreted as indicating that riboflavin could be excreted into the upper part of the small intestine, and that when downward movement toward the ileum and large intestine was prevented by ligature, further excretion against a high concentration gradient in the upper small intestine was prevented, or possibly that reabsorption from the small intestine kept pace with absorption.

The second series was designed to ascertain whether the riboflavin reached the duodenum by way of the bile of intestinal epithelium. Ligatures, placed so that they did not interfere with the blood supply, subdivided the gastrointestinal tract into separate compartments: stomach, duodenum, jejunum, ileum, cecum, and large intestine. The influx of bile was completely eliminated. At autopsy riboflavin crystals were found in large quantities in the duodenum and jejunum, but no detectable amounts were found elsewhere. It was concluded that the biliary tract plays no part in the excretion.

In a third experiment the riboflavin was introduced directly into various parts of the intestinal tract and it was found that it could be readily absorbed from the lower part of the small intestine and then excreted into the upper part. Another series showed that riboflavin is not efficiently adsorbed in the large intestine but is rapidly destroyed. By removal of the entire gastrointestinal tract it was shown that the excretion and destruction of riboflavin must occur largely through the function of renal and intestinal tissue.

Sarett and Perlzweig (*J. Nutrition* **25,** *173 (1943)*) studied the effect of protein and B-vitamin levels of the diet upon the tissue content and balance of riboflavin (and nicotinic acid) in young male rats by means of complete balance experiments. Previously they had shown that the urinary excretion of riboflavin in rats, and nicotinic acid by dogs, bore an inverse relationship to the level of protein intake. Riboflavin and nicotinic acid were inferred to be concerned with protein anabolism. Weight curves and urine values were followed and carcass and liver analyses made. Four diets, fed ad libitum, were used: high protein (40 per cent)-high vitamin, high protein-low vitamin, low protein (8 per cent)-high vitamin, and low protein-low vitamin. The riboflavin and nicotinic acid content per gram of rat carcass, excluding the liver, was the same for all four diet groups. The riboflavin and nicotinic acid contents per gram of liver were low in the animals on both the low protein diets, regardless of the vitamin intake. The low riboflavin levels in the liver on the low protein diets, with the normal value for the remainder of the carcass, suggest that the carcass levels are maintained at the expense of the liver. Protein therefore appears to be necessary for retention of riboflavin in the liver.

Riboflavin utilization, therefore, appears to be dependent not only on its intake but also on the protein level of the diet. When adequate protein is supplied, the optimum intake for adult rats is equivalent to 0.9 micrograms per 1000 calories of food—the accepted level for the adult human being. For the growing young the requirement appears to be roughly three times this amount.

IRON AND IRON SALTS IN FLOUR ENRICHMENT

Nakamura and Mitchell (*J. Nutrition* **25,** *39 (1943)*) point out that difficulties may be encountered in meeting the federal specifications regarding the addition of iron to flour as part of the enrichment program. Iron salts which are not insoluble may cause adverse reactions such as rancidity in the flour. On the other hand, if the iron of any preparation is not readily assimilated by the body, the purpose of its addition is defeated. A study was made, therefore, of the degree to which the several iron salts commonly incorporated in flour (iron phytate, sodium iron pyrophosphate, and ferrum reductum) were utilized for the production of hemoglobin. The rates of hemoglobin regeneration were measured in rats which had previously been made anemic by milk feeding, and were compared with those induced by ferric chloride.

The amount of iron given daily to each animal was 2 mg. because this quantity permits considerable but not maximal regeneration of hemoglobin in nutritional anemia according to Smith and Otis (*J. Nutrition* **13,** *573 (1937)*). The iron in sodium iron pyrophosphate and in ferrum reductum was as well utilized as was the iron in ferric chloride. The iron in ferric phytate, however, proved to be definitely inferior and stimulated hemoglobin formation only about half as much as did ferric chloride. Iron retention data based on carcass analyses indicated that the absorption of iron had been less from ferric phytate than from the other iron salts. Therefore, the limiting factor which made ferric phytate an inferior therapeutic agent was apparently its poor absorption.

Since reduced iron was well utilized, the authors suggest that the nutritive value of some foods may be enhanced by the iron added as a contaminant during processing, particularly as a result of the abrasion of iron-containing grinding mills. They found, for example, that whole corn with an original iron content of 0.0035 per cent may possess an iron content of 0.0061 per cent after it has been ground through a cutting mill, and as much as 0.0125 per cent after grinding through a burr mill.

Widdowson and McCance (*Lancet* **I,** *588 (1942)*) obtained some evidence that human subjects utilize the iron of white bread more completely than the iron of 92 per cent extraction bread. This may be related to the fact that iron phytate occurs only in the outer layers of the wheat kernel while the iron in patent flour is largely inorganic, and originates to a considerable extent from the milling process. Nakamura and Mitchell found that the iron of whole wheat is between 50 and 66 per cent as available for hemoglobin regeneration as is the iron of ferric chloride.

Of interest from the experimental standpoint was the care taken to equalize the intake of "mineralized milk" between pair mates by adjusting the supply in accordance with the appetite of the poorer eater. This "mineralized" milk added to skim milk powder formed the anemia-producing diet. Such precaution was imperative since a variable intake of milk, even though the test source of iron was constant, would produce a variable rate of hemoglobin production. With this equalization of intake the sex differences reported by several investigators disappeared entirely.

If the food enrichment program is to be of maximal value, it must be supported by evidence such as that presented here. This study emphasizes that it is not the chemical constituents alone that are important but their utilization by the body that gives them nutritional value. Caution, however, should be exercised in assuming that human subjects would necessarily behave as did the anemic animals in assimilating iron. Clinical experience has demonstrated that 1.5 to 3.0 g. of reduced iron per day are required to give the same therapeutic result as is produced by 1 g. of iron as iron ammo-

nium citrate or by 0.2 g. of iron as ferrous sulfate. While iron phytate is probably absorbed poorly by man as well as by the rat, it does not necessarily follow that the human subject absorbs reduced iron as easily as ferric chloride.

THIAMINE IN HUMAN MILK

Although the nutritional requirements of normal infants are not established with any great degree of arithmetical precision, they are known sufficiently well so that the practical feeding of the healthy baby has been reduced to standardized procedures. For the vast majority of infants human milk or cow's milk suitably modified forms the basis of the diet. When supplemented with sources of vitamin C and of vitamin D (and with carbohydrate), the usual cow's milk modifications seem adequate in meeting the requirements for growth and development of the infant. It is possible that iron and thiamine may need special consideration, but as yet the requirements for these two substances have not been thoroughly worked out, for the younger infant especially. It is conceded that it is desirable to supplement the diet of the older infant with food preparations which are rich in iron. As a rule no special attention is given to vitamin B_1 but Jeans, (*J. Am. Med. Assn.* **120,** *913 (1942)*), who has contributed a review of the principles of feeding healthy infants and children has stated that "early supplement with thiamine containing foods is desirable."

A number of years ago it was suggested that evaporated milk may have lost some thiamine in its heat processing and during subsequent storage. Many babies are reared on evaporated milk feedings; hence interest has been aroused in whether the loss of thiamine in evaporated milk is significant. Studies have shown that evaporated milk when diluted with an equal volume of water on the average may be expected to contain just about as much thiamine as does boiled pasteurized milk, and considerably more than average human milk. In a recent paper Knott and collaborators (*J. Pediatrics* **22,** *43 (1943)*) have shown that earlier reports of the thiamine content of human milk probably were in error, being too low. Using the microfermentation method of assay of Atkin, Schultz, and Frey, examination was made of 111 samples of breast milk taken from 50 different women. In harmony with the observations of others it was found that colostrum, the first secretion of the mammary glands, contains practically no thiamine. As true milk begins to be secreted the thiamine content gradually is increased, but it requires three or more weeks for the milk to acquire its typical concentration. The average value in mature milk was 15.1 micrograms to each 100 ml. of milk. Women who were able to nurse their infants without the necessity of supplementary feeding of the baby had milk containing 20.1 micrograms to each 100 ml., whereas women who had to supplement the intake of their infants, or who were weaning them, had an average thiamine content of 9.3 micrograms in each 100 ml. of milk. The administration of thiamine intramuscularly during labor or orally after delivery caused a rapid rise in the thiamine content of the milk, so that the time required for attaining maximum thiamine concentration could be reduced from three or four weeks to nine days.

These values are higher than those previously reported, even by the same group of workers, who earlier found from 3 to 18 micrograms, or an average of 9 micrograms of thiamine in each 100 ml. of human milk. With the same technic, using both microbiologic and thiochrome methods, pasteurized milk was found to contain from 18 to 35 micrograms, and averaged 26; boiled pasteurized milk averaged 24 micrograms in

each 100 ml. Evaporated milk after dilution with an equal volume of water had from 13 to 27 micrograms, or an average of 19 micrograms of thiamine in each 100 ml. of human milk. Kendall (*J. Pediatrics* **20,** *65 (1942)*) also has reported low values, from 0 to 9 micrograms in each 100 ml. of human milk. The average values reported by other investigators have been from 5 to 11 micrograms. These low values may be owing in part to faulty technic in the extraction of the vitamin from the samples used for analysis, but it seems clear that cow's milk contains on the average about twice the concentration of thiamine as breast milk, and average evaporated milk is almost as high. Human milk of course has long been considered with considerable justification to be the best possible food for the human infant. Results such as those reported by Knott and collaborators indicate that this view may not be correct as far as thiamine is concerned, unless the mother is receiving an adequate intake of this dietary essential.

Jeans, in discussing the subject, has called attention to the different distribution of calories in human milk and in cow's milk modifications. There are more calories from carbohydrate in the latter and, because thiamine is concerned with carbohydrate metabolism but not with the metabolism of fats, there may not be much discrepancy between the two types of milk in their ability to meet the thiamine requirement of the infant.

Knott and collaborators have tried to estimate the thiamine requirement of the infant. Nine infants who were receiving unsupplemented human milk, which contained about 20 micrograms of thiamine in 100 ml., were given oral test doses, consisting of 100 micrograms of thiamine to each kilogram of body weight, and the urine subsequently voided was collected and examined for its thiamine content. These infants excreted less thiamine than did 8 infants who presumably were receiving higher intakes of thiamine either because they had been receiving evaporated milk or because the mothers were receiving supplements of thiamine. The greater excretion of the latter group was interpreted as an indication that these infants did not need to retain as much of the test doses of thiamine which were administered.

Although the data presented are relatively meager the authors have suggested that the thiamine requirement of the normal infant is about 0.20 mg. daily, or as a practical standard, about 40 micrograms of thiamine for each kilogram of body weight. This value is identical with that suggested by the Food and Nutrition Board of the National Research Council. It can be met only if the milk contains 20 micrograms or more of thiamine in each 100 ml., or if other sources are supplied.

The interesting report of the Chicago investigators, like so many fruitful studies, appears to pose more questions than it answers. Our knowledge of the thiamine requirement of the infant is hardly more than a guess, although data such as those reported show that it is an intelligent guess. The work does emphasize the desirability of giving attention to the diet of the lactating mother.

It would seem wise, because of the paucity of evidence now available, to interpret these reports as indicative of the desirability of maintaining the nutritive status of the lactating mother, rather than as evidence of the supposed imperfection of human milk.

B-VITAMINS IN TUMOR TISSUE

The occurrence of B-vitamins in all forms of living matter is strong presumptive evidence of their essential nature in vital processes. The exact function of many of these

vitamins is still unknown, but all appear to be necessary for the growth of young animals, and at least three, thiamine, niacin, and riboflavin, are necessary components of intracellular enzyme systems. The two properties, stimulation of growth and coenzyme activity, make it logical that the B-vitamins should be considered in connection with the cancer problem, since tumors differ from most adult tissues not only in their greater rates of growth but also in their relatively high capacity to glycolyze under aerobic conditions.

Accordingly, Williams and associates have made a survey of the concentration of several of the B-vitamins in a variety of tumor types, and have compared the results with those of similar determinations made on representative normal tissues. Microbiologic methods of assay were employed throughout.

Riboflavin assays were made by Pollack, Taylor, Taylor, and Williams (*Cancer Research* **2,** *739* (*1942*)). The tumors studied included samples of the Walker carcinosarcoma and of a primary hepatoma induced in rats by p-dimethylaminoazobenzene; a transplantable adenosarcoma and a primary sarcoma induced in mice by methyl cholanthrene; and 22 samples of various human cancers. Normal tissues from the three species served as controls.

The riboflavin content of the various tumors was found to be relatively constant and most of them contained between 2 and 3 micrograms of riboflavin per gram of moist tissue (extreme ranges 1.6 to 7.4 micrograms). In contrast the normal tissues fall into two groups. Brain, lung, spleen, and muscle ranged from 1.6 to 4.4 micrograms per gram; sample of liver, kidney, and heart ranged from 7.8 to 51.0 micrograms of riboflavin per gram. Thus, while liver tumors, for instance, contained much less riboflavin than normal liver, the riboflavin content of the tumors did not differ significantly from that of a great many normal tissues. A similar distribution was encountered for niacin by Taylor, Pollack, Hofer, and Williams (*Cancer Research* **2,** *744* (*1942*)). Most of the tumors contained from 18 to 29 micrograms of niacin per gram, extreme ranges 13 to 59, while the normal tissues ranged from 18 to 179 micrograms per gram. In other words, for this vitamin as for riboflavin, the tumor values were relatively consistent, relatively low, and well within the range for normal tissues. The same general tendency was also observed for pantothenic acid (*Cancer Research* **2,** *752* (*1942*)) and for biotin by Pollack and co-workers (*Cancer Research* **2,** *748* (*1942*)).

The induced liver tumor contained less of every one of the vitamins studied than did the original normal liver. But this decrease may well have been a result rather than a cause of the carcinogenic process, since normal liver is much higher in these vitamins than most of the other tissues of the body. Apparently as a liver tumor develops, it tends to acquire those concentrations of vitamins characteristic of tumors in general.

Taylor, Pollack, and Williams (*Science* **96,** *322* (*1942*)) regard the uniformity in vitamin content for neoplasms of various origins as indicative of a basic similarity in the metabolism of all tumors. In this connection it would be interesting to compare the vitamin content of a sarcoma with the connective tissue from which it is arising. Presumably the vitamin content of connective tissue is low and hence the vitamin content might actually increase as the tumor develops. But while it is highly probable that the B-vitamins are important in tumor metabolism, it still remains to be determined whether the observed deviations in vitamin content are in any way connected with the critical biologic differences between normal and tumor tissues.

EMERGENCY LIVESTOCK NUTRITION

Restricted feeding and emergency rations are in order for the nation's herds and flocks during 1943. The housewife is keenly aware of the limitations in variety and amount of food available for the family table, but few people appreciate the problems of the farmer in providing an adequate nutrition program for his livestock. Although many livestock feeds are not rationed, they are available in very limited amounts. The feeding problems of the livestock producer have been presented by Hunter (*J. Am. Vet. Med. Assn.* **102,** *217 (1943)*) and Miller (*J. Am. Vet. Med. Assn.* **102,** *300 (1943)*).

The livestock industry is faced with the problem of increasing production with limited amounts, per animal unit, of many of the feed ingredients which, in times past, have been considered essential to a sound and economical feeding program. The most serious shortage is that of protein, particularly sources of animal protein such as fish meal, tankage, meat scrap, and dried milk. These shortages are both real and apparent. The total livestock population has increased over former years, a situation which would make even a normal supply of protein inadequate. In addition, restrictions on production and importation have decreased the available supplies of such products as fish meal and tankage. The production of soybeans has been increased tremendously but this has not fully solved the protein shortage because of the bottleneck imposed by the lack of adequate processing equipment which is required to render soybean protein suitable for livestock feeding.

It is apparent that sufficient carbohydrates are available for the livestock feeding program but the total supply of these must now be diminishing. The supply of fats for domestic livestock is now less than that previously available, owing to other war needs and to processing methods which now remove more fat from potential livestock feed. There is no apparent shortage of calcium for livestock feeding but a restricted supply of bone meal indicates that it will be difficult in many instances to provide phosphorus in adequate amounts. No satisfactory solution is apparent.

With respect to vitamins, perhaps the most critical situation is that of vitamin A (carotene) from plant sources such as alfalfa. Unfavorable climatic conditions adversely affected high quality alfalfa production in 1942, with the result that present supplies of vitamin A from this source are limited.

Realization of the seriousness of the situation has resulted in the formulation of emergency feeding programs by the Committee on Animal Nutrition of the National Research Council, and the adoption of a voluntary agreement by leaders in the feed industry, designed to ensure the best use of all available protein for the various classes of livestock.

A practical solution of the protein problem is to discontinue liberal protein feeding in all instances. Rations with 12 to 18 per cent of protein, depending on the type of roughage, are in order for dairy animals. It is recommended that whenever animal protein is available for swine it is to be fed only to weanling pigs and breeding stock. Protein quality may be maintained by the use of oil meals as supplements to the cereal grains. The fullest use of all available pasture will be helpful in conserving the supply of concentrates and in providing vitamin A. Sunshine can be depended on for some vitamin D and will help to reduce the requirement of minerals for bone development.

It would seem to be inevitable that a restricted feeding program for swine will result in slower growth and less economical pork production. It is questionable whether

dairy production will be seriously impaired if present expectations of supplies of dairy feeds are met.

It remains to be seen, also, whether increased susceptibility to disease or definite symptoms of deficiencies will result from these wartime feeding practices. The American Veterinary Medical Association has recognized the seriousness of the situation and has appointed a Special Committee on Nutrition (*J. Am. Vet. Med. Assn.* **102,** *124 (1943)*) to coordinate the efforts of veterinarians in recognizing and treating cases of nutritional failure.

VITAMIN C AND EXPERIMENTAL HEPATIC DAMAGE

The importance of nutrition to the maintenance of normal hepatic function is receiving major emphasis in current physiologic investigations. Much of the recent work relating the metabolism of vitamin A and the B-complex to liver function has already been covered in Nutrition Reviews. The practical medical value of these researches is indicated by repeated suggestions that the clinical course of hepatic cirrhosis may be influenced favorably by dietary measures. There is also abundant evidence that high carbohydrate and protein rich diets may offer partial protection against hepatotoxins like chloroform and arsphenamine while fat may be injurious (*Miller and Whipple, Am. J. Med. Sci.* **199,** *204 (1940)*; *Messinger and Hawkins, Ibid.* **199,** *216 (1940)*). Less attention, however, has been given to the effect of ascorbic acid on the liver.

Murakami demonstrated several years ago that when guinea pigs were fed a diet deficient in ascorbic acid, their livers detoxified less well and excreted smaller amounts of bile and bile pigment. These functional changes were reversed when the deficiency was relieved (*Jap. J. Gastroenterol.* **11,** *1 (1939)*). Ratsimamanga observed that liver glycogen in scorbutic guinea pigs was reduced to about one fifth of normal (*Travail humain* **7,** *303 (1939)*). More recently Beyer has studied the protective action of ascorbic acid on hepatic damage produced by hydrazine (*Arch. Int. Med.* **71,** *315 (1943)*). This latter substance was chosen as the hepatotoxin for the experiment because its toxic effect is relatively selective for hepatic cells. Two groups of guinea pigs were placed on a diet of Purina mixed rabbit chow which contains a negligible amount of vitamin C. Each of the animals in one group was injected daily with 30 mg. ascorbic acid subcutaneously; the pigs in the second group received no supplemental therapy. After about sixteen days, the animals in the latter group ceased to gain weight. The guinea pigs in both groups were then given injections of 25 mg. of hydrazine sulfate per kilogram of body weight on two successive days. Forty-eight hours later the experiment was terminated and the livers of the animals were examined grossly, microscopically, and chemically for their fat content. Beyer had previously determined that in pigs fed the Purina ration, with or without supplemental ascorbic acid, (a) the weight of the liver in relation to body weight, (b) the percentage of dry matter in liver, and (c) the hepatic fat content, were substantially the same as for "normal" guinea pigs (ration not stated). The livers of vitamin C deficient animals did contain more fat, but the increase was slight. After the hydrazine, however, the livers from the animals that had received vitamin C were grossly normal while those from the scorbutic pigs were yellow, pale, and heavier in relation to body weight by 37.5 per cent. The cytologic evidences of parenchymal damage were minimal in the group to which the supplement had been given, but extensive and extreme in the deficient animals. Finally, the livers of the

scorbutic group contained 50.3 per cent more lipoid material than did those of the group given vitamin C. Beyer's data convincingly demonstrate that ascorbic acid protected guinea pigs from the hepatotoxic effect of hydrazine to a significant degree. The degree of vitamin depletion in the "unprotected" (control) group was severe, however.

The mechanism of the protective action is not clear. It is possible that ascorbic acid may exert its effect indirectly by permitting normal metabolism of carbohydrate and protein. Beyer's experiments do not indicate that excess ascorbic acid would necessarily have additional protective value for an animal whose vitamin C reserves are normal. It would be interesting to know this since there have been clinical reports that vitamin C has therapeutic value in the treatment of patients who exhibit a sensitivity to organic arsenicals and to gold. It is at least possible that the fundamental protective mechanism is the same in these clinical experiences as in the experiments here reported.

SALICYLIC ACID HYPOPROTHROMBINEMIA

The hemorrhagic manifestations in cattle with sweet clover disease and those in rats induced by administration of 3,3′-methylenebis (4-hydroxycoumarin) are known to be concerned with the metabolism of vitamin K. This vitamin prevents the hemorrhagic manifestations and the associated hypoprothrombinemia. When, however, the coumarin derivative is administered to various species of animals, a decrease in prothrombin cannot be detected even by the most sensitive tests until twelve to twenty-four hours after its administration. Essentially the same lag in response occurs even if the drug is given intravenously, and spectrographic analysis has shown that it is absent in the blood stream before the maximum intensity of response is obtained. 3,3′-methylenebis (4-hydroxycoumarin) does not affect the clotting power of blood or plasma *in vitro*.

Of several possible explanations for these facts, Link and associates (*J. Biol. Chem.* **147,** *463 (1943)*) postulated that the coumarin derivative might have to undergo a metabolic degradation before it acts on the prothrombin mechanism. Other work at the Madison laboratory by Huebner and by Stahmann showed the quantitative chemical degradation of 3,3′-methylenebis (4-hydroxycoumarin) to salicylic acid. A general investigation of a variety of compounds showed that the active hypoprothrombinemic agents, by virtue of their chemical structures, might all yield salicylic acid or a closely related acid upon degradation. An investigation was therefore made of the possible hypoprothrombinemic activity of salicylic acid itself. A positive and rapid production of decreased prothrombin levels by this relatively simple substance, and the antagonistic action of vitamin K, should lead to a better understanding of the mechanism of the vitamin action on prothrombin formation.

Rats were used as the experimental animals and two rations employed. The first, referred to as the stock grain ration, consisted of corn, soybean meal, linseed oil meal, alfalfa leaf meal, skim milk powder, butterfat, and salts. The second, called the artificial ration, was composed of casein, yeast, Wesson oil, cod liver oil, cerelose, and salts. The latter diet was very low in vitamin K, the former contained considerably more, but both were adequate for maintenance of the rats. Salicylic acid was administered either in the ration or intravenously. The prothrombin time was measured with a plasma concentration of 12.5 per cent, an experimental condition which allows the detection of milder degrees of hypopro-

thrombinemia than tests with more concentrated plasma.

At levels of 2, 10, and 100 mg. of salicylic acid daily, rats on the stock grain diet developed a mild hypoprothrombinemia only on the higher dosage in a period of sixty days. On the low vitamin K ration, those rats receiving 100 mg. of salicylic acid daily developed a severe hypoprothrombinemia in twenty days. At a 300 mg. level the average survival time of 6 animals was only ten days, during which time hemorrhagic manifestations developed which were entirely similar to those found in rats after administration of 3,3′-methylenebis (4-hydroxycoumarin). Rats on the artificial ration developed a temporary mild hypoprothrombinemia even after single oral doses of 10, 25, or 100 mg. of salicylic acid, and a more marked change after intravenous administration of sodium salicylate.

Preliminary treatment of the animals with 2-methyl-1,4-naphthoquinone, a compound with vitamin K activity, exerted a complete protective action against the effect of salicylic acid. The protective action of vitamin K undoubtedly explains the difference in the sensitivity of the animals on the different diets to salicylic acid. The difficulty noted in producing hypoprothrombinemia by salicylic acid in rabbits and dogs is probably also related to the fact that experimental vitamin K deficiency is difficult to produce in these animals.

Approximately ten times as much salicylic acid is required to produce the same quantitative effect as the methylenebis-coumarin. The investigators suggested that much of this difference may be due to the more rapid distribution and elimination of salicylic acid. As a consequence, salicylic acid shows a more rapid response, occurring as early as three to six hours after parenteral administration.

Dilution experiments and other experimental changes of the prothrombin time test have convinced the investigators that the prolonged clotting times caused by salicylic acid are due primarily to an induced hypoprothrombinemia entirely similar to that caused by the coumarin derivative. Salicylic acid, like the coumarin derivative, has no effect on the clotting process *in vitro*.

Link and associates believe that the results established may have a direct bearing on salicylate therapeutics in man. They call attention to medical reports that high dosage of salicylic acid in rheumatic fever may cause hemorrhage from the mucous membranes in certain individuals; that in cases of methyl salicylate poisoning, the principal changes are vascular with hemorrhagic lesions; and that there is increasing evidence that extensive use of aspirin may produce severe gastric hemorrhage, so-called "aspirin hematemesis." It must be emphasized that only large doses of salicylates are implicated in these hemorrhagic conditions.

Although no details are given, it is reported that two independent clinical investigators have been able to reproduce the results of the hypoprothrombinemic action of salicylates in man "even when the diets are not restricted."

The studies of Link and associates not only add to our knowledge of vitamin K and its action but also evoke a new approach to the mechanism of the toxic, and perhaps the therapeutic, action of drugs.

NUTRITIONAL INADEQUACIES IN PREGNANCY

The relative importance of diet during pregnancy has been the subject of several excellent studies published within the past two years. Among the first to appear was that of Ebbs, Tisdall, and Scott (*J. Nutrition* **22**, *515 (1941)*). This group was interested in the fact that, while the deaths of infants under 1 year of age have been mar-

kedly reduced in the past twenty-five years, the number of deaths in the first few weeks of life have been altered little—and in many instances unexplainable deaths have occurred. Their study was planned to determine what effect, if any, prenatal diets might have on the outcome of pregnancy and on the health of the infant during the early months of life. The influence of diet was found to be striking, and suggests the need for the obstetrician to consider the dietary habits of his patients and to give sound nutritional advice as a routine part of prenatal care.

These workers showed that mothers receiving good diets were better obstetrical risks; their general mental attitude was better, and labor was shorter. The details of this study are tabulated and are impressive, both regarding the mother's health and effects on the infant in the first months of life. The authors considered the blood hemoglobin, vitamin C, and phosphatase; the mother's condition during the prenatal period, labor, and convalescence; her ability to nurse the infant; and the condition of the infant at birth and at the age of 6 and 12 months.

During the prenatal period there were more cases of anemia, toxemia, and threatened miscarriage in the poor diet group. The total number of complications in this group was almost double that in the group of women whose diets were not only basically adequate but who, in addition, received supplementation. During pregnancy the needs for protein, calcium, iron, and the vitamins are apparently increased. The differences in the nutritional needs of the nonpregnant, pregnant, and lactating woman are expressed in the recommended daily allowances of the Food and Nutrition Board of the National Research Council (*Nutrition Reviews* **1,** *164 (1943)*).

Williams and Fralin (*Am. J. Obst. Gyn.* **43,** *1 (1942)*) likewise studied the effect of the nutritional status on pregnancy and reached similar conclusions. More recently a survey was made by Bethell, Blecha, and Van Sant (*J. Am. Dietet. Assn.* **19,** *165 (1943)*) on the nutritional inadequacies in pregnancy as they are correlated with the incidence of anemia. This study confirms the earlier reports, particularly on the incidence of anemia in pregnancy.

Bethell and associates remind the reader that the most prevalent, and from a therapeutic standpoint the most important anemias, are those which result from inadequacies of the substances needed for blood formation. Some of these substances (iron, copper, protein, portions of the B-complex, and the "extrinsic factor" of Castle) are supplied by the diet; others such as the "liver principle" must undergo intermediate metabolic change before being utilized in the manufacture of blood cells. Nutritional inadequacies arise not only from the diet lacking these essential constituents but also from failure to absorb normally or utilize them. The defects of diet itself are exacerbated by pathologic states and by certain physiologic conditions such as growth, menstruation, pregnancy, and lactation, and especially when they follow in rapid sequence.

These authors report a field survey of dietary habits and hematologic values of pregnant women carried out in two rural counties in Michigan. The data were collected by repeated interviews in the homes, and by blood examinations throughout gestation and for variable periods up to a year thereafter. Home interviews made possible the economic and social classification of these women on which the comparisons were based. Of the 484 women included, 246 lived on farms and 238 lived in small towns. Ninety-two were able to obtain the food recommended without difficulty, 326 could manage by planning their food budget with care, but 66 needed financial assistance in order to meet even the basic essentials of the nonpregnant woman.

Their data indicate that 60 per cent of the pregnancies were in the age group between 20 and 30. It was the first pregnancy

for 40 per cent of the women, the second for 26 per cent, and the third for 15 per cent. Analysis of weight gain shows a tendency to progressive overweight with each pregnancy. These authors suggest that part of the responsibility for this unwanted weight change rests on physicians who urge their patients to eat excessively in order to increase milk production, on the assumption that food quantity is the major factor involved.

The effect of instruction in dietary habits was most encouraging and indicates that during pregnancy one has an opportunity to alter food habits, not only for an immediate purpose but for the future benefit of both the patient and her family. The tables show, for example, that it was possible to increase the calcium intake of 75 per cent of the women to over 1.2 g. daily and in 16 per cent more to raise it to a level of 0.7 to 1.2 g. Similar improvement is shown in the phosphorus, and vitamins A, D, C, B_1, and B_2 intake.

The incidence of anemia among the women was 25.4 per cent. Less than 5 per cent had from 12 to 15 mg. of iron daily, around 55 per cent had from 8 to 11 mg., while 15 per cent had below 8 mg. The percentage of hypochromic anemia, as would be expected, was correlated with these iron intake levels.

The association of inadequate intake of protein of high biologic value to macrocytic anemia had previously been reported by Bethell and associates. These new data substantiate the earlier findings. When the the average daily protein intake was 79 g. or over, there was approximately a 2 per cent incidence of anemia, in the 60 to 79 g. group it rose to 7 per cent, in the 40 to 59 g. group to 13 per cent, while those receiving less than 40 g. of protein daily showed a 27 per cent incidence of anemia.

This study again emphasizes the specific relationship between food intake and the nutritional state, especially as it pertains to hematologic values in pregnancy and the significant positive effect of dietary instruction. The authors recommend that means be devised for providing dietetic instruction and hematologic examination as a routine and important part of prenatal care—a recommendation in which they are by no means alone.

DEFICIENCY DISEASE IN PATIENTS WITH MEDICAL DISORDERS

Recent dietary surveys have indicated that the frequency of malnutrition in the United States is high. That present and threatened food shortages may aggravate existing dietary inadequacies is obvious. However, there has been reluctance on the part of many persons, including physicians, to accept these evidences for the prevalence of malnutrition as valid since deficiency diseases are not too commonly observed. If the dietary analyses could be supplemented with chemical evaluation of vitamin reserves, they would be much more convincing, but this is as yet not practical because methods are either not sufficiently specific or are too difficult to apply to large population groups. One other possible way to check the validity of the nutritional surveys would be to determine whether persons with poor diets tend to develop deficiency diseases when their nutritional requirements are increased during periods of illness. This has been done by Goldsmith who studied 200 patients admitted to the medical wards of the Charity Hospital in New Orleans (*South. Med. J.* **36,** *108 (1943)*). These subjects varied from 5 to 82 years of age, but 60 per cent were over the age of 40 years. They were so selected that there were 50 white males, 50 white females, 50 Negro males, and 50 Negro females; the subjects in each group represented consecutive admissions to the hospital.

Two studies indicate that the average

diet of the population from which these patients were drawn is poor. In one, the eating habits of 5776 grade and high school students were analyzed from records of all food consumed during one week (Coco and associates, *Mimeographed Publication of the Nutrition Laboratories, College of Agriculture, Louisiana State University, Baton Rouge, Louisiana (1943)*). As judged by standards recommended by the Food and Nutrition Board of the National Research Council, only 11.2 per cent of the white and 3.3 per cent of the Negro children received a diet adequate in all essential food factors. The diets were fair or "border line" in 59.6 per cent of the white and 38.1 per cent of the Negro students, poor and definitely deficient in one or more essential nutrients in 29.2 per cent of the white and 58.6 per cent of the Negro children. A similar survey of dietary records of 1082 Farm Security Administration families in Louisiana made during 1939 and 1940 showed only 1.22 per cent which could be classified as good, while 69.28 per cent were fair and 29.49 per cent poor (Grigsby, McBryde, and Davis, *Mimeographed Publication of the Nutrition Laboratories, College of Agriculture, Louisiana State University, Baton Rouge, Louisiana (1941)*).

In her study, Goldsmith tabulates only the occurrence of riboflavin and niacin deficiency in the 200 patients, although she states that deficiency of vitamin A, thiamine, and ascorbic acid was also observed. The symptoms and signs accepted for the diagnosis of ariboflavinosis and aniacinosis were (frequency of each is recorded in parenthesis): sore tongue and mouth (25), chronic diarrhea (17), nervousness and irritability (54), visual disturbances (80), nasolabial sebacious plugs (65), cheilosis (34), changes in the tongue (111), pellagrous dermatitis (9), thick pigmented skin over bony prominences (90), vascularization of cornea (18), and conjunctival congestion (44). Diagnosis of the deficiency state was made only in the presence of characteristic signs, or of a combination of highly suggestive signs and symptoms. Riboflavin deficiency was thought to exist in 44 subjects, niacin deficiency in 11, and both in 24. Fifty-four of the subjects were considered as "probably deficient" and 67 showed no evidence of deficiency. There were fewer Negroes than white patients with deficiency disease, but the reason for this racial difference was not clear. The incidence of clinically evident deficiencies in the various medical diseases encountered is listed in the accompanying table:

Disease	Total patients	Patients with deficiency
Gastrointestinal diseases	22	14
Heart disease without failure	20	11
Heart disease with congestive failure	22	14
Hypertension, arteriosclerosis, and nephritis	14	8
Cerebral hemorrhage and thrombosis	5	3
Infections (fever usually present)	45	28
Blood dyscrasias	9	4
Diabetes mellitus	9	7
Hyperthyroidism	3	3
Malignancy	18	15
Cirrhosis of liver and hepatitis	6	6
Chronic alcoholism	3	3
Pellagra (endemic)	2	2
Miscellaneous	22	15

The fact that deficiency disease became clinically recognizable in at least 79 of these 200 patients when they developed various organic medical diseases which either caused a decreased assimilation of foodstuffs or increased metabolic demands supports the idea that their nutritional status had previously been in precarious balance. It strongly suggests that the dietary surveys made on population groups from which they were drawn were correct when they suggested that the diets were poor. While the diagnoses of mild riboflavin and niacin deficiencies seem to have been made on adequate clinical evidence, cognizance must be taken of the tremendous personal element

involved when diagnoses are made solely on the basis of signs and symptoms. Goldsmith mentions having used a niacinamide type of tolerance test, but it is not clear that any of the persons showing evidence of niacin deficiency by this method were actually patients in her group of 200. Discussion of the results of specific therapy with niacin and riboflavin is also made, but no direct data are recorded for the response to specific therapy of the subjects included in this analysis. Finally, it is unfortunate that no mention is made of the time of year during which these patients were observed since the season may influence the incidence of endemic riboflavin and niacin deficiencies even when they are precipitated by other medical diseases.

BIOTIN AND CANCER

The demonstrated relationship of many of the essential dietary factors to cellular respiration makes a study of their connection with tumor metabolism a logical step. Of the dietary factors which may be concerned, biotin in particular has received attention. West and Woglom (*Cancer Research* **2,** *324 (1942)*) found that malignant tumors differed widely from the corresponding adult tissue in their biotin content, some being very rich in this vitamin (*Nutrition Reviews* **1,** *51 (1942)*). This did not, of course, indicate that the presence of biotin led to the development of malignancy; it may become concentrated in certain tissues as the consequence, rather than the cause of the tumor growth. However, du Vigneaud and co-workers (*Science* **95,** *174 (1942)*) observed that the addition of biotin to diets which prevented in rats the development of hepatomas induced by the feeding of butter yellow appeared to break down the protection those diets afforded. This naturally raised the question, "If tumor tissue requires exceptionally large quantities of biotin, is it possible to reduce the growth of neoplasms to a greater extent than that of normal tissues by withholding biotin from patients suffering from cancer?"

An attempt to answer this question was made by Rhoads and Abels (*J. Am. Med. Assn.* **121,** *1261 (1943)*). Studies were carried out in 2 patients, one with inoperable cancer of the breast which had extended through the chest wall and had metastasized to the liver and axillary and supraclavicular lymph nodes; the other, a patient with chronic lymphocytic leukemia. Rhoads and Abels attempted to induce biotin deficiency in these 2 patients by feeding a diet low in its content of biotin and by giving raw, frozen egg white, dried egg white and, later, an avidin concentrate. For twelve weeks the diet furnished 60 micrograms of biotin per day. Later this was reduced to 46 micrograms. From 1000 to 1200 units of avidin per day were fed at first; later the quantity was increased by 500 to 750 units, and still later by 1000 to 1250 units. Their attempt to induce biotin deficiency in man was encouraged by the report of Sydenstricker and co-workers (*J. Am. Med. Assn.* **118,** *1199 (1942)*). The latter fed 1000 units of avidin in the form of crude egg white as well as a diet containing relatively small quantities of biotin to 4 human volunteers and observed, in four to twelve weeks, the development of a maculosquamous dermatitis, grayish pallor of the skin, atrophy of the lingual papillae of the tongue, mild depression, extreme lassitude, sleeplessness, loss of appetite, and other changes. The biotin excreted in the urine of these experimental subjects decreased 3.5 to 7.3 micrograms in twenty-four hours as compared with an excretion of 29 to 62 micrograms in persons on an ordinary diet (*Nutrition Reviews* **1,** *18 (1942)*).

The experiment of Rhoads and Abels was continued for thirty weeks. The patients

received no treatment other than dietary, as described above. Neither subject developed the clinical syndrome described by Sydenstricker *et al.*, and the course of their neoplastic disorders was not altered from what would have been expected in the usual course of events in the diseases in question. It is also important to note that the biotin content of the urine ranged from 6 to 132 micrograms and later 15 to 270 micrograms in one patient and from 21 to 70 micrograms in the second patient. In normal human subjects consuming ordinary diets Oppel (*Am J. Med. Sci.* **204,** *856 (1942)*) reported excretion of 14 to 111 micrograms of biotin per day with the majority of the findings ranging between 20 and 50 micrograms.

These studies are important in several respects. They indicate, in the first place, the difficulty of inducing biotin deficiency in man even when a potent source of avidin, a substance which combines with biotin and is known experimentally to promote the development of biotin deficiency in rats, is supplied in large quantities. Like Oppel, Rhoads and Abel found that more biotin was excreted in the urine than was received in the diet despite the simultaneous ingestion of large amounts of avidin. The failure to reproduce the observations of Sydenstricker and co-workers is also noteworthy but does not necessarily reflect on their accuracy.

The approach to the treatment of cancer by reducing the available amounts of substances necessary for tissue respiration may fail, just as have so many other promising methods of treatment of this recalcitrant disorder. The studies reported by Rhoads and Abels, however, do not provide the final answer to the question they raise, as they themselves indicate, since they did not succeed in inducing biotin deficiency. Furthermore, it is possible that the biotin requirement of different types of malignant diseases may vary considerably and the dietetic approach might succeed in certain types of carcinoma even though it fails in other forms. One also wonders whether an alteration in the biotin available for tumor tissues can possibly affect their susceptibility to the effects of radiation. The road to the defeat of cancer is a long and circuitous one. Unfortunately, its end still seems invisible.

VITAMIN RETENTION IN COOKED MEATS

Meat has long been recognized as one of the best dietary sources of protein, phosphorus, and iron. It was not possible, however, until suitable assay methods could be developed, to evaluate meats as dietary sources of the various members of the vitamin B-complex. It is now known that animal tissues serve as excellent sources of both fat soluble and water soluble vitamins. Lean pork, for example, is one of the richest natural food sources of water soluble thiamine. The extensive literature on the vitamin content of meats has been reviewed by Waisman and Elvehjem ("The Vitamin Content of Meat." *Burgess Publishing Company, Minneapolis, Minnesota. (1941)*).

Now that meat has been included in the the food rationing program it is increasingly important to have as much authoritative information as possible relative to the various factors affecting its vitamin content. One phase of the problem that has received too little consideration is that of cooking. Lane, Johnson, and Williams (*J. Nutrition* **23,** *613 (1942)*) describe experiments in which the thiamine content of raw and fried ham was studied, using rat curative and thiochrome methods of assay.

Thiochrome assays of fried ham gave substantially lower results than those obtained by rat curative tests. At first this was thought to be due to insufficient extraction of thiamine, although digestion of the cooked meat with sulfuric acid or

proteolytic enzymes failed to increase the thiochrome results appreciably. It was concluded that thiamine becomes partially bound, during cooking, "through the reaction of the pyrophosphoric acid group with the amino group of protein amino acids and that this linkage is split with great difficulty." This conclusion was further substantiated by dehydrating the fresh ham by boiling in xylene until the samples were free of water and fat. This low temperature dehydration reduced the thiamine, as determined by the thiochrome method, about 41 per cent. Portions of the same dehydrated sample showed no reduction in thiamine when measured by the rat curative method. Both methods were in substantial agreement when applied to fresh uncooked ham, and the rat curative method on fried ham yielded results identical with those obtained on fresh ham by the same technic. The conclusions are important and interesting and should be confirmed by further and more extensive studies.

Previous to the work just described, Waisman and Elvehjem had conducted studies, also reported in "The Vitamin Content of Meat," in which several types of meat had been cooked by various methods. In these studies thiamine was determined by the rat growth method. No effort was made to determine cooking temperatures inside the meats. It was concluded that there is a destruction of thiamine in most cooking processes although in two frying experiments (beef and ham) no thiamine destruction could be noted. Results varied from no destruction at all to as much as 57 per cent. Losses due to roasting, stewing, and boiling were consistently high and varied with time of cooking.

Riboflavin determinations, made by the microbiologic method, indicated that while some destruction may occur in meat during cooking, the losses were less marked than those recorded for thiamine. Here again, results were highly variable indicating that care should be taken in drawing conclusions.

A very recent study relative to the retention of vitamins in meat during cooking is that of McIntire, Schweigert, Henderson, and Elvehjem (*J. Nutrition* **25,** *143* (*1943*)). These workers preface their discussion by stating that accurate information on the subject of vitamin destruction in meats during cooking is very meager and that the present work was undertaken with the view of standardizing cooking methods, which had not been done in the previous studies conducted in their laboratory.

Sampling of pork ham and loin was standardized as to anatomical position, thickness of cut, and thickness of fat. Standard cooking procedures, as developed by the U. S. Department of Agriculture and representatives of State Experiment Stations, were employed. Fresh loin cuts were roasted, braised, or broiled; ham cuts were roasted or boiled. Time of cooking varied with size and thickness and internal temperatures were recorded. Fresh and cooked samples were ground in an electric meat grinder to assure homogeneity and were stored in dark glass at −4° C. Duplicate moisture and fat determinations were made on all samples. All drippings from cooked samples were heated for 15 minutes with 0.1 N sulfuric acid and the aqueous layer was preserved for vitamin estimations. Thiamine determinations were made by the Hennessy thiochrome method (*Cereal Chemists Bull.* **2,** *25* (*1942*)) with modified extraction procedures. Riboflavin was determined by the microbiologic method of Snell and Strong (*Ind. Eng. Chem.* (*Anal. Ed.*) **11,** *346* (*1939*)), and nicotinic acid was measured by the microbiologic method of Snell and Wright (*J. Biol. Chem.* **139,** *675* (*1941*)), using a 4 per cent sodium hydroxide digestion. While seven different series of samples were completed, not all the data are included in the published paper.

The per cent of thiamine retained in the various cuts of meat varied as follows: roast loin 64 to 70; braised loin 49 to 54; broiled loin 55 to 79; roast ham 62 to 80; boiled ham 72 (for all samples). The per cent of the total thiamine retained in the meat plus the drippings varied as follows: roast loin 67 to 76; braised loin 71 to 73; broiled loin 57 to 85; roast ham 66 to 84; boiled ham 75 to 82. In the case of riboflavin, the per cent retained ranged between 70 and 85, and again the total riboflavin retained by the various cooking methods in meat plus drippings was much greater, ranging from 85 to 100 per cent. The percentage of nicotinic acid retained in cooked meat ranged between 63 and 74 in braised loin, and from 81 to 98 in roast ham. When data concerning drippings were included the total nicotinic acid retained was from 80 to 100 per cent.

The authors point out that it is not possible to compare their recent data directly with previous data from their laboratory owing to the fact that more fat was left in the samples just described. This tends to give somewhat lower vitamin concentrations than were reported in earlier experiments, in which the tissues were freed from fat.

They call attention to variations in vitamin content of different cuts from the same loins and hams and postulate that different muscles may vary in the amount of stored vitamin. No marked differences could be noted in actual destruction of vitamins in the various processes of roasting, braising, or boiling, but the loss from drippings appears to be considerably greater in braising than when the meat is roasted or boiled. As much as 24 per cent of the original thiamine may be found in drippings from braised loin samples while drippings from broiled loin and ham cuts average only about 6 per cent. Thiamine loss in the drippings from roast loins and hams is negligible. Likewise in the case of nicotinic acid, the greatest loss in the drippings occurs with braised meat. Owing to the ability of the meat proteins to retain riboflavin, there is a relatively small loss (5 to 15 per cent) of this vitamin in the drippings.

These studies are most helpful in emphasizing the necessity of standardizing cooking procedures and pointing out practical ways and means to preserve the maximum nutritive value of meats, and emphasize the important role that meats, particularly pork, can play in helping to satisfy our daily vitamin requirements.

ENVIRONMENT AND VITAMIN REQUIREMENTS

Environmental conditions may have to be considered in determining vitamin requirements. Mills earlier indicated (*Am. J. Physiol.* **133,** *525 (1941)*) that rats kept at 91°F. had a considerably higher thiamine requirement than others kept at 65°F., and now reports a similarly increased requirement of choline and pyridoxine at an increased environmental temperature (*Arch. Biochem.* **1,** *73 (1942)*). This study was concerned with the effect of environmental temperature on the requirement of certain of the B-complex vitamins. Experimentally, 3 week old male white rats were raised in air conditioned chambers, one chamber maintained at 68°F. and another at 91°F. and 70 per cent relative humidity. Their basal diets contained sucrose, casein, corn oil, a salt mixture, and a purified vitamin complement. The effect of a given vitamin was then estimated by its removal from the diet and replacement in graded quantities. The adequacy of the supplement was then judged on the basis of growth responses. Food consumption was also recorded.

In the investigation of choline, 6 groups

of rats, 4 rats in each group, and at each temperature, were given the following choline supplements: 0, 0.37, 0.75, 1.5, 3.0, and 5.0 g. of choline per kilogram of diet. A total of five weeks' observation showed that the weight gain of the rats maintained at 68°F. was maximal at a level of 0.75 g. of choline per kilogram of diet and nearly as good at the 3.0 and 1.5 g. levels. Both the weight gains and food consumption decreased at higher levels of choline intake. In distinct contrast, the rats maintained in the tropical moist heat showed an increased growth and food consumption over the whole range of choline supplementation studied. The results at the two temperatures were strikingly different. On the choline-free diet, 5 out of 8 rats died at the lower temperature, 4 out of 8 at the warmer temperature. Growth of the surviving members was satisfactory after the danger period of acute hemorrhagic nephritis had been passed.

In the pyridoxine investigations, day old white leghorn cockerels were used. Chicks maintained at the lower temperature were kept at 78° to 80°F. for ten days before being placed in a 70°F. chamber. The other group was maintained at 91°F. In each group pyridoxine levels of 0, 1, 2, 4, and 8 mg. per kilogram of diet were used. At 70°F. chick growth was essentially as good at a level of 1 mg. as at higher levels, but at 91°F. optimal growth was not reached until a level of 4 mg. per kilogram of diet was reached. In the colder room 14 out of 15 chicks died on the pyridoxine-free diet, in the warm room 10 out of 15. In the hot chamber at pyridoxine levels below 4 mg. per kilogram of diet there was a frequent occurrence of polyneuritis. Preliminary observation showed that as much as 12 mg. of thiamine per kilogram of diet was necessary to prevent this, but the polyneuritis syndrome was a complicated one and requires further study.

In the case of pantothenic acid, there was a sharp optimum in growth and food consumption at a level of 6 mg. per kilogram of diet, but this was the same in rats maintained at either temperature. Likewise in the case of biotin and riboflavin, no significant differences were found in the rats maintained at different temperatures. The negative results with these vitamins serve to emphasize, in a sense, the distinct differences found with choline and pyridoxine.

It was pointed out that paired feeding experiments, desirable as they might seem, could not be carried out since the cold room animals starved to death in a week when allowed only the food intake of those maintained at the higher temperature. Growth at the higher temperature "was accomplished with less wastage of calories for maintenance of body temperature, and was most efficient in both heat and cold at the optimal vitamin concentration." In the case of choline and pyridoxine the requirements were larger whether calculation was based on vitamin concentration in the diet, on actual daily intake, or on food intake per unit of body weight.

A summary of experiments, all of which are not yet reported, indicates that "rat requirements for optimal growth are twice as high at 91°F. as at 68°F. for thiamine and pyridoxine and over five times as high for choline." These experiments are interesting and thought provoking, but much more work is necessary before results of this kind will be widely accepted. There is no evidence that the findings can be applied directly to human nutrition.

HEMOGLOBIN AND PLASMA PROTEIN PRODUCTION

The most recent report from Whipple's laboratory (*J. Exp. Med.* **77**, *375* (*1943*)) offers much of interest to the clinician as well as to the student of protein metabolism.

Using dogs from their well known anemia colony, Robscheit-Robbins, Miller, and Whipple fed these animals a basal diet low in protein and adequately supplemented with iron as well as vitamins. Repeated withdrawal of blood reduced the hemoglobin and plasma protein levels and, with protein as the only apparent limiting factor, the role of various sources of protein and the value of amino acid mixtures for the production of hemoglobin and plasma protein were studied. By determining total blood volume and calculating total hemoglobin and plasma protein production, interesting observations concerning the body economy were made.

The value for hemoglobin and plasma protein production of the following was studied: dog plasma, dog hemoglobin, hemoglobin digests, beef serum digests, casein digests, a mixture of the amino acids necessary for rat growth (Rose), and cystine plus choline. All of these were utilized by the animals. In order of decreasing effectiveness for hemoglobin production they fell roughly as follows: dog plasma given intravenously, dog hemoglobin given intraperitoneally, casein digest by mouth or by vein, beef serum digests orally or intravenously, amino acid mixture given by mouth, hemoglobin digests given intravenously or orally, and finally cystine plus choline given orally. For plasma protein production the order was not quite the same. In this respect dog plasma took the lead very easily but was followed by casein digests, dog hemoglobin given intraperitoneally, beef serum digests, hemoglobin digests given intravenously, and in the lowest category the amino acid mixtures and hemoglobin digests given by mouth.

It is noteworthy that all the substances were used to a greater extent for hemoglobin production than for plasma protein replacement. However, since the quantity of hemoglobin is roughly three times the amount of plasma protein in the dog this is not altogether surprising. It is of interest that dog plasma and beef serum digests when given orally (but not when given intravenously), as well as the casein digests, promoted plasma protein production somewhat more than the other substances tested. Curiously, when cystine replaced methionine in the amino acid mixture the balance also turned somewhat more favorably to plasma protein production. These investigators also noted that the production ratio of the amino acid mixtures, when calculated according to their theoretical protein equivalent, compared favorably with the best dietary proteins.

The implications of these studies are numerous. It is evident that the plasma proteins contribute freely to hemoglobin production and that there is a ready interchange between plasma proteins and hemoglobin in both directions. A "dynamic equilibrium" exists. In the author's words, "this all adds up to a remarkable fluidity in the use of plasma protein or hemoglobin which can contribute directly to the body protein pool from which are evolved, without waste of nitrogen, the needed proteins, whether hemoglobin, plasma protein, or tissue proteins."

We have learned that it is not wise to assume that observations in one species necessarily apply to another, but the clinician should be stimulated by these studies to give more attention to the importance of protein in hemoglobin production. It will be important to determine whether it is possible to demonstrate clear benefit from the use of protein in certain types of anemia, such as has been shown to be the case in the use of iron and of highly purified liver extracts in the treatment of chronic hypochromic anemia and the nutritional macrocytic anemias, respectively. There are many forms of anemia in the treatment of which neither iron nor liver extract is of value.

What use can be made, by proper digestion, of red cells discarded in blood banks

as a source of protein for hemoglobin and plasma protein formation remains to be seen. These studies contribute also to the immediate problem concerning substitutes and supplements for plasma and whole blood in the treatment of shock and of conditions in which absorption from the gastrointestinal tract is impaired.

VITAMIN C SATURATION

The analysis of blood and urine for vitamin C, with or without the test dose procedure, has yielded valuable information concerning the nutritional status of the individual with respect to this vitamin. There have been many modifications of the test dose procedure, but most have attempted to gain maximum simplicity for application to survey work. Successive blood samplings or collection of twenty-four hour samples of urine have not met popular approval in such work.

L. J. Harris and co-workers (*Lancet* **I,** *642 (1942)*) have outlined a simple saturation test which consists in the administration of 700 mg. of ascorbic acid daily per 140 pounds of body weight, until such time as the urinary excretion of the vitamin reached 50 mg. in a one hundred thirty-five minute period of urine collection, commencing at the fourth hour. Harris (*Nature* **151,** *21 (1943)*) tested this method on boys in an orphan home in England. Their basal diet was carefully checked for its vitamin C content and graded levels of ascorbic acid were added. Boys on supplementary levels of 45 to 75 mg. of ascorbic acid, who were then given the saturation test dose procedure, gave first day responses. A level of 40 mg. per day gave a second day response, while 25 mg. gave a third day response upon application of the test. Those on lower intakes showed three to five day responses and scorbutic patients required seven to ten days for saturation. The data were interpreted to mean that the number of days beyond the second may be taken as an index of the relative deficit in the past intake.

With these data established, it is desirable to know what factors may commonly affect the vitamin C status of a group. Harris and Olliver (*Nature* **151,** *22 (1943)*) followed the influence of seasonal fluctuations in the supply of available sources of vitamin C to the residents of an orphanage. The highest daily intakes of 42 to 55 mg. daily were found during the summer months and the lowest, 19 to 24 mg. daily, during the spring, while winter intakes were intermediate in quantity. These differences were reflected in the time required for saturation, necessitating one day in the summer and two days during the winter. The difference was probably accounted for on the basis of a change from old to new potatoes and to the increased use of garden produce.

The simplified saturation test has been used extensively by the British armed forces to check on the vitamin C nutrition of its men. Atkins (*Nature* **151,** *21 (1943)*) applied the test, with certain modifications, to 1200 men. The general usefulness of the test was indicated by the finding that only two to three daily doses of 0.75 g. were necessary to saturate those already well supplied with ascorbic acid while eight or more doses were required for those bordering on the scorbutic state. Marked individual variations were noted within groups and possible reasons were given for the differences. Severe muscular exercise appeared to produce different effects on the vitamin C status of the subjects. Ascorbic acid may be lost in perspiration, and hence differences in the tendency of individuals to perspire could also produce variations within a group.

The simplified saturation test, with due appreciation of the factors which may affect its validity, should be a useful method for survey work.

NOTES

Letters to the Editor

Dear Sir:

In the April 1943 number of Nutrition Reviews (p. 168) there was an article on fatty livers. In the introductory paragraph credit for observing the lipotropic action of choline, was given to Junkersdorf and Kohl. I have looked up this paper, which had escaped my attention before, and I feel that no data worth serious consideration were presented in favour of the action of choline as a dietary factor or, in fact, of any effect of choline on the fatty liver of starvation in dogs. The difference between the fat content of the liver of the choline-treated animals and the values which were used as controls, is certainly not significant considering the number of animals used. Furthermore the controls were not described in this paper at all but reference was made to some results which may or may not have been obtained under comparable conditions. They were taken from an article published five years previously by Junkersdorf. The choline was given intravenously or subcutaneously and the profound pharmaco-dynamic effect might have been responsible if positive results really had been secured. Furthermore all the work in rats shows that choline, as a dietary factor, has no effect on the fatty liver of starvation and although this possibility has not been exhaustively tested in dogs, one would expect the same results. In some preliminary tests in 1933, we found no effect of choline on the starvation fatty livers in pups.

A number of my colleagues have gone over the paper and the unanimous opinion is that the data do not in any way justify the conclusion that choline given under the conditions which are described, has any effect on deposition of fat in the liver.

Sincerely yours,
C. H. Best,
Professor of Physiology,
University of Toronto,
Toronto, 5, Canada.

Dear Sir:

Upon looking through the May number of Nutrition Reviews I note a small error. Because I am sure you desire readers to help check on the accuracy of the Reviews I am calling it to your attention.

On page 221, in a review of "Synthesis of vitamins in the digestive tract" it is stated that Guerrant, Dutcher, and Tomey used cecetomized rats in their studies on this problem, and it is implied that their conclusions concerning the importance of the cecum were largely based on data obtained from such animals. Actually they cecetomized two rats. Experimental data were obtained from only one because one of the two failed to survive the operation. Although their work was valuable, Griffith was the first to employ significant numbers of cecetomized animals in such studies.

Sincerely yours,
Harry G. Day,
Associate Professor of Chemistry,
Indiana University,
Bloomington.

Dear Sir:

The third sentence, in the third paragraph of the first column on page 195, of the May number of "Nutrition Reviews" sounds to me a little odd. If the slit lamp was not used, examination could hardly have been done according to the technique of Dr. Sydenstricker, unless Dr. Sydenstricker gave some special directions which are different from those of the technique which he ordinarily uses. (*He did.—Editor.*)

I am making this comment not as a serious criticism but just as a matter of interest. I am, however, a little bit concerned about the writings and discussions of some who apparently do not have any very clear idea of the real nature of the conditions which Dr. Sydenstricker and his associates originally described.

Let me congratulate the Editorial Staff on the Reviews.

Sincerely yours,

John B. Youmans,
Professor of Medicine,
Vanderbilt University,
Nashville, Tenn.

Postwar Food Production in Europe

Postwar food production in Europe will present a serious problem, according to a report by Allied agricultural experts which is now being studied by the British Technical Advisory Committee on Agriculture (*Science* **97,** *215 (1943)*). The decline of livestock in enemy-occupied countries is estimated to approximate 11,000,000 cattle, 3,000,000 horses, 12,000,000 pigs, and 11,000,000 sheep. Milk and meat production have declined to appallingly low figures and will continue to decline as feeding stuffs become even more scarce and as more animals are requisitioned by the enemy.

Many years will be required to bring animal production back to prewar levels and the lack of draught animals will handicap future crop production. Emphasis is laid on the necessity of livestock conservation and the preservation of the best breeding stock, in order to meet the postwar food problem. Tractors will have to be used in many areas where draught animals are no longer available. As a result, the Committee has recommended that a school be established in Britain to train Allied nationals in agricultural engineering in order that mechanized services can be set up in many agricultural areas, just as soon as the war is over.

Milk Curds

It is common practice in infant feeding to modify cow's milk to give a soft, finely divided curd in the stomach since the belief is widely prevalent that soft curd milk is more digestible. This belief has not been supported in the case of 10 to 13 year old children by the work of Hadary, Sommer, and Gonce (*J. Dairy Sci.* **26,** *259 (1943)*). They studied the gastric emptying time by roentgenograms and by frequent fluoroscopic examinations, and tested homogenized pasteurized whole milk, chocolate milk, diluted evaporated milk, and the usual pasteurized milk. The stomach or colonic emptying time was found to be independent of the curd tension of the milk. This conclusion cannot be extended a priori to individuals with gastrointestinal abnormalities or to infants.

Thiamine Stability in Wheat Germ

At the National Research Laboratories, Ottawa, Canada, Pearce (*Canad. J. Res.* **21,** *57 (1943)*) has studied the development of rancidity in commercial wheat germ and the effect of storage on the thiamine content. The moisture was adjusted to known amounts above and below the normal content of 12.5 per cent and the resulting specimens were stored at different temperatures. It was found that wheat germ keeps better at low moisture content (8.0 per cent) and at low temperatures, and that storage life is lengthened by packing in nitrogen and by compressing the germ into blocks. Students of nutrition will be interested in his observation that the thiamine content, as determined by the thiochrome method, did not vary even though the wheat germ might become rancid. Thus, in one series of experiments, the thiamine content was initially 14.2 micrograms to the gram, and after two weeks 15.5 micrograms to the gram, where the value remained constant for twenty-six weeks. Although the experimental details are not presented, the author calls attention to a number of observations which have led him to consider that protein hydrolysis may be an important factor—more important than fat hydrolysis—in the deterioration of wheat germ as well as of other food products, such as egg yolk, during storage.